

Evaluation of hepatoprotective activity of *abelmoschus moschatus* seed in paracetamol induced hepatotoxicity on rat

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Abstract: Paracetamol (PCM) is widely used as analgesic and antipyretic drug, but at high dose it leads to undesirable side effects, such as hepatotoxicity. This study gives the information about hepatoprotective activity of *abelmoschus moschatus* seed extract against paracetamol and ethanol induced hepatotoxicity. Paracetamol induced hepatotoxicity was evaluated by an increase ($P < 0.05$) in serum AST, ALT, ALP activity and bilirubin level. Paracetamol hepatotoxicity was manifested by an increase ($P < 0.05$) lipid peroxidation, depletion of reduced glutathione (GSH) and catalase activity in liver tissue. Administration of ethanolic as well as aqueous plants extract [300mg/kg body weight of rat] protects the paracetamol induced lipid peroxidation, restored altered serum marker enzymes and antioxidant level towards normal. The results indicate hepatoprotective activity of all studied plants extract against paracetamol induced toxicity. Ethanolic extract was found more significant than the aqueous extract.

Keywords: ethanol, hepatoprotective, paracetamol, silymerine, whister rats

I. INTRODUCTION

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals (e.g., microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. [Friedman, *et al*, 2003]

Paracetamol is widely used as antipyretic and analgesic drugs, causes hepatotoxicity if taken in the excess amount of dose. The mechanism of hepatotoxicity is firstly cause the necrosis of Centilobular hepatocytes and followed by lipid per oxidative degradation of glutathione and produces cell necrosis in liver due to the formation of intermediate oxidative product of paracetamol (N-acetyl-P-benzoquinoneimine). (Kapur V. *et al*, 1994)

The *abelmoschus moschatus* seed belong to family Malvaceae. It contains sitosterol b. total phenol, flavonoids which are responsible for antioxidant, antimicrobial and free radical scavenging activity. [[Rastogi, Mehrotra, 1991, Mir Z Gul1, *et al*, 2011,]

It also shows protective mechanism in Respiratory troubles and asthma Antispasmodic Itch [I.M. Liu, *et al*, 2010]

II. MATERIAL AND METHODS

Drugs and Chemicals:

Paracetamol was purchased from the local market of Bhopal in the tablet dose form by the PARACIP-500 brand name product of "CIPLA" on 28/01/2012. Silymarin was purchased from in the capsule dose form LIMARIN® 70 by the brand name product of Serum Institute of India Ltd on 28/01/2012. Other chemicals Petroleum ether, Ethanol are also used.

Instruments used- Weighing machine, Grinder, Soxhlet, Analytical balance, and Water bath.

Plant Material:

1 kg of dried seed of *Abelmoschus moschatus* was collected from the local market of Bhopal on 28/01/2012. *Abelmoschus moschatus* seed was authenticated by the Head of Department of botany Dr.zia Ul Hasan Professor of Safia College of Science Bhopal. Plant authentication no. is 346/Bot/Safia/2012 on the date-14/02/2012. Seed was grind by the Grinder mixture and extract was collected from that powder with the help of Soxhlet apparatus.

Animals:

Male *Wistar* rats weighing 200 ± 20 g were used for this study. The animals were kept in polypropylene cages and maintained at $25 \pm 5^\circ\text{C}$ and $60 \pm 5\%$ humidity under 12 h light/dark cycle. The animals were allowed free access standard pellet diet and water. The animal experiment was performed according to the guidelines laid by Institutional Animal Ethical Committee (IAEC).

Experimental protocol:

Animals were divided into five groups of six rats each and treated orally as below for 14 days.

Group-1: Normal control, given water daily for 14 days (o.d).

Group-2: Standard, Paracetamol (500mg/kg body weight) +30% ethanol + Silymarin (25mg/kg body weight) p.o. for 14 days (o.d).

Group-3: Paracetamol control, given water daily for 14 days followed by single dose of paracetamol (500mg/kg body weight) +30% ethanol p.o. for 14 days (o.d).

Group-4: Ethenolic Extract (300mg/kg body weight) + Paracetamol (500mg/kg body weight) +30% ethanol p.o. dose for 14 day (o.d).

Group-5: Aquous Extract (300mg/kg body weight) + Paracetamol (500mg/kg body weight) +30% ethanol p.o. dose for 14 days (o.d).

Animals were sacrificed under light chloroform anesthesia 24-h after the last dose. Blood was collected by retro orbital plexus puncture.

Biochemical Assays:

Serum marker enzymes of liver function: Serum was separated by cooling centrifugation at 3000 rpm at 4°C for 10min and used for measurement of various biochemical markers such as SGOT, SGPT, (AST and ALT) activities, alkaline phosphatase (ALP) activity, and total bilirubin using commercially available kits.

Statistical analysis:

The values were expressed as mean \pm SD. Statistical analysis and comparison between the groups was performed by one way analysis of variance (ANOVA)

Difference between unexposed and exposed (with or without treatment) with a *p*-value < 0.05 was considered significant.

Result

Table1. Pharmacognostical screening of powder

S. no.	Component	Result
1.	Moisture content	11.14%
2.	Ash value	15%

Table2. Pharmacognostical screening of extract

S. no.	Test	Observation	
		Alcoholic	Aquous
1.	Flavonoid	+ve	+ve
2.	Totalphenolic compound	+ve	+ve
3.	Alkaloids	_ve	_ve
4.	Saponines	_ve	_ve
5.	Glycosides	_ve	_ve
6.	Tannins	_ve	_ve
7.	Resins	+ve	_ve
8.	Nitrogenous compound	+ve	+ve

Table 3. Acute oral toxicity test

S. no.	Dose in ml	No. of animals	Observation
1.	300 mg/kg	3	All animal survived
2.	600mg /kg	3	All animal survived
3.	1200mg/kg	3	All animal survived
4.	1600 mg /kg	3	All animal survived
5.	2000 mg/kg	3	All animal survived

Table 4. Evaluation of Antioxidant Activity of *Abelmoschus moschatus* seed extract against DPPH scavenging.

S. no.	Concentration (µg/ml)	% inhibition	
		Aqueous	Alcoholic
1	100	32.32	55.23
2	120	29.00	52.86
3	140	26.22	50.41
4	160	23.43	47.00
5	180	21.43	43.10
6	200	19.10	41.11

Table 5. Evaluation of effect of *Abelmoschus moschatus* on serum SGOT, SGPT, ALP, T.B in paracetamol + ethanol induced hepatotoxicity.

S. no.	Group	BLOOD SERUM BIOCHEMICAL DATA(MEAN ±SEM)			
		SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)	T.B(mg/dl)
1.	Control	67.5±3.35	76.66±2.47	26.66± 3.073	1.41± 0.305
2.	Standard	75.83±2.386	85.83±4.362	35.83±2.713	2.0±0.18 2
3.	-ve control	109.16±2.713 a***,b***	202.5±4.787 a***,b***	97.5±5.881 a***,b***	6.0±0.8062 a***,b***,
4.	Eth-extract	90±2.887 a***,b*,c**	104.16±2.386 a***,b*,c***	56.66±6.54 a**,b*,c***	3.08±0.327 c***
5.	Aq-extract	100±2.887 a***,b***	107.5±4.425 a***,b**,c***	67.5±4.233 a***,b***,c* *	3.83±0.3073 a**,b*,c*

The data were analyzed by one way ANOVA followed by Tukey's multiple comparisons Test.

Each values represents the mean ± SEM; n=6, *p<0.05, **p<0.01, ***p<0.001

a- Significant difference as compare to normal control group

b- Significant difference as compare to negative control group

c- Significant difference as compare to standard group

d- Significant difference as compare to ethanolic group

Graph 1. Evaluation of effect of *Abelmoschus moschatus* on serum SGOT, SGPT, ALP, T.B in paracetamol+ ethanol induced hepatotoxicity.

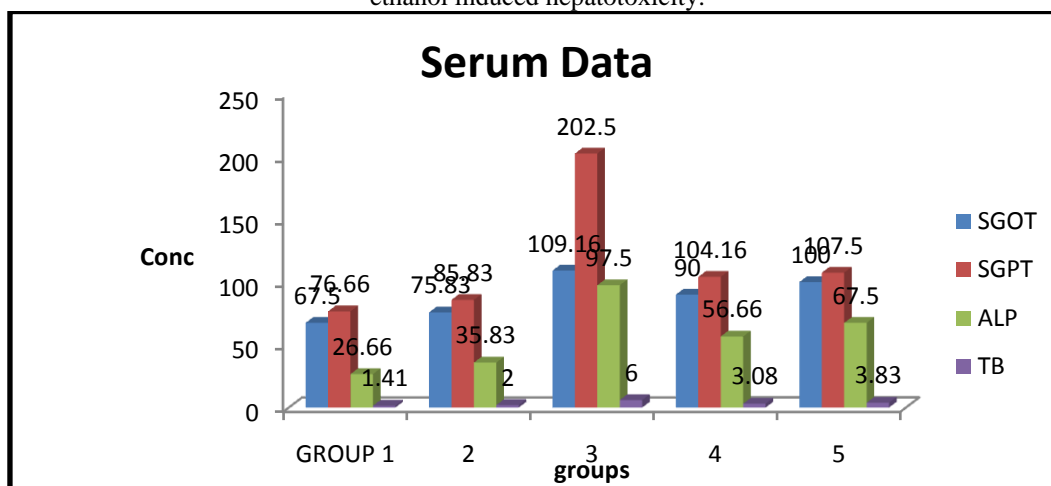


Table 6. Evaluation of effect of *Abelmoschus moschatus* on serum TG and TC, in paracetamol+ ethanol induced hepatotoxicity.

S.N.	GROUP	TG (mg/dl) Mean ± SEM	TC (mg/dl) Mean ± SEM
1	Normal control	134.23 ± 0.40	102.14 ± 0.46
2	Standard	150.32 ± 0.34 a***, b***	136.22 ± 0.21 a***, b***
3	Negative control	335.35 ± 0.51 a***	220.41 ± 0.20 a***
4	Test-2(Alcoholic)	201.34 ± 0.50 a***, b***, c***, d***	170.11 ± 0.43 a***, b***, c***, d***
5	Test-1(aqueous)	171.33 ± 0.41 a***, b***, c***	167.34 ± 0.32 a***, b***, c***

The data were analyzed by one way ANOVA followed by Tukey multiple comparisons Test.

Each values represents the mean ± SEM; n=6, *p<0.05, **p<0.01, ***p<0.001

- a- Significant difference as compare to normal control group
- b- Significant difference as compare to negative control group
- c- Significant difference as compare to standard group
- d- Significant difference as compare to aqueous group

Graph 2. Evaluation of effect of *Abelmoschus moschatus* on serum TG and TB in paracetamol + ethanol induced hepatotoxicity.

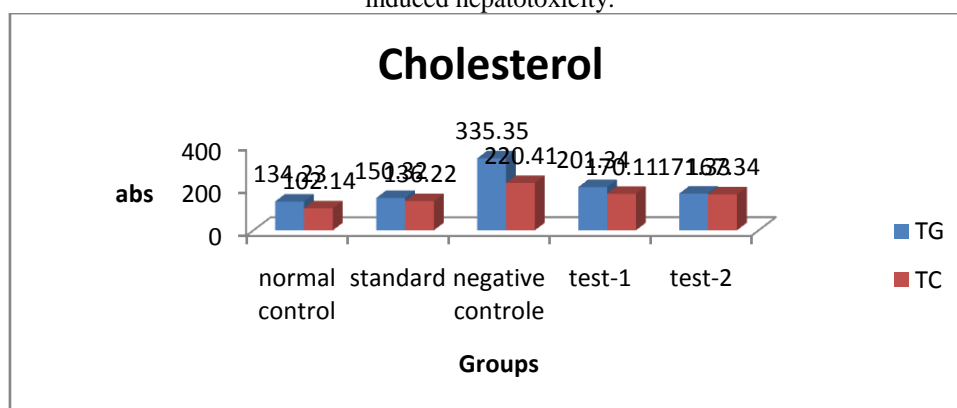


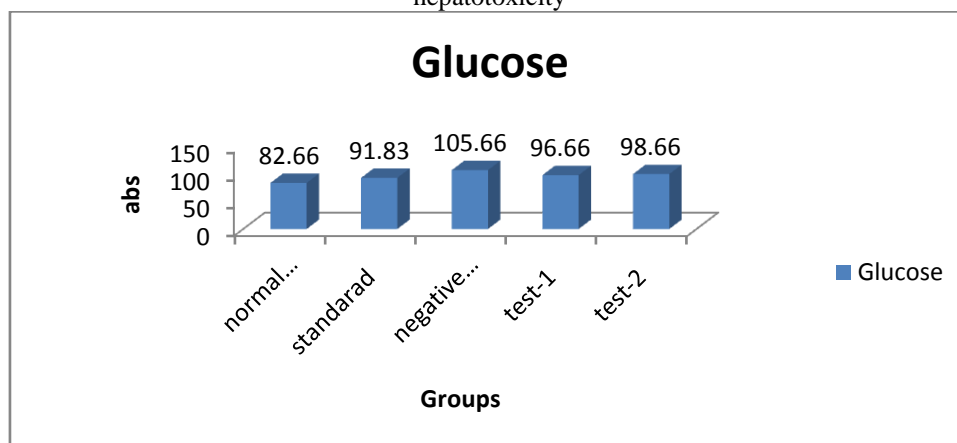
Table-7. Evaluation of effect of *Abelmoschus moschatus* on Blood Glucose in paracetamol+ ethanol induced hepatotoxicity.

S. no.	Group	Blood Glucose(mg/dl)(Mean \pm SEM)
1	Normal control	82.66 \pm .95
2	Standard	91.83 \pm .83 a***,b***
3	Negative control	105.66 \pm .55 a***
4	Test-2(alcoholic)	96.66 \pm .90 a***,b***,c***
5	Test-1(aqueous)	98.66 \pm .71 a***,b***,c**

The data were analyzed by one way ANOVA followed by Tukey multiple comparisons Test. Each values represents the mean \pm SEM; n=6, *p<0.05, **p<0.01, ***p<0.001

- a- Significant difference as compare to normal control group
- b- Significant difference as compare to negative control group
- c- Significant difference as compare to standard group
- d- Significant difference as compare to aqueous group

Graph-3. Evaluation of effect of *Abelmoschus moschatus* on Blood Glucose in paracetamol+ ethanol induced hepatotoxicity



5. Histopathological study

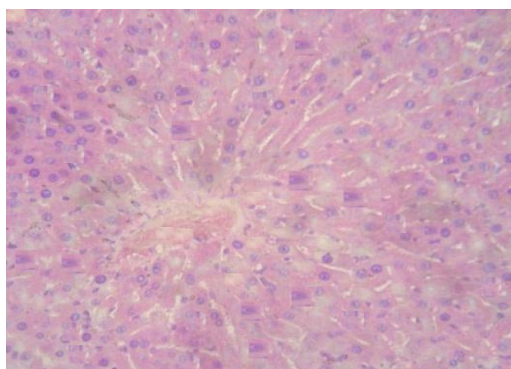


Fig-normal control

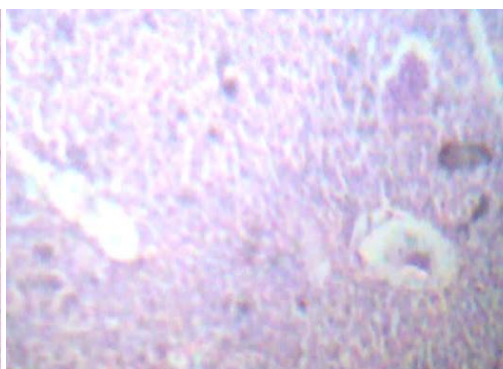


Fig-Negative control

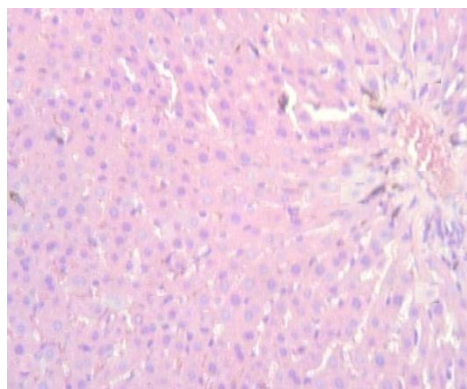


Fig-Standard



Fig-Aqueous extract

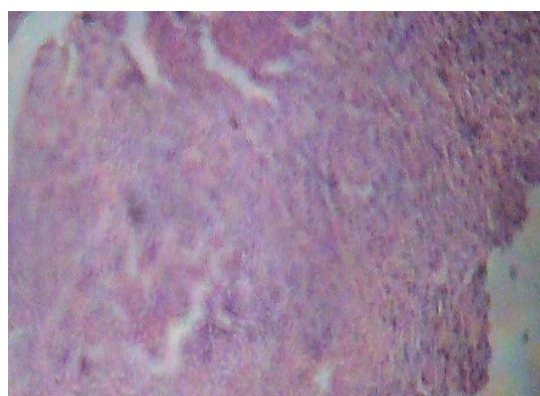


Fig- Alcoholic extract

III. DISCUSSION

The object of my project is to study the Hepatoprotective activity of *Abelmoschus moschatus* seed extract. Instead of presence of various types of allopathic drugs for Hepatoprotective, herbal drugs are preferred in the market due to its safety.

The *Abelmoschus moschatus* is cultivated in most places of India. It is usually shown in March-April. Seed are black in colour and irregular in shape and having bitter and acrid taste.

From the extraction of *Abelmoschus moschatus* seed alcoholic extract was found-2.4% and aqueous extract was found-1.8%. The alcoholic extract of seed contains Flavonoid, Total phenol compounds, Nitrogenous compound and Rasines while while aqueous extract contains only Flavonoid, Total phenol compounds, and Nitrogenous compound other compound such as Alkaloids, Saponine, Tannins, Glycosides are absent. The seed contains 11.14% moisture and 15% Ash value. (Table-6.1, 6.2)

The hepatoprotective activity is studied by the paracetamol + ethanol induced hepatotoxicity in either sex of Wister albino rats. The protocol was taken for 14 days and drugs were administered orally. The degree of liver damage is determined by investigation of various parameter i.e.-SGOT, SGPT, TB, and ALP, and other parameter which assist these study are Total Cholesterol, Triglycerides, and blood Glucose levels etc, and finally Histopathological studies. (Table-5.9.2)

Paracetamol is widely used as antipyretic and analgesic drugs, causes hepatotoxicity if taken in the excess amount of dose. The mechanism of hepatotoxicity is firstly cause the necrosis of Centilobular hepatocytes and followed by lipid per oxidative degradation of glutathione and produces cell necrosis in liver due to the formation of intermediate oxidative product of paracetamol (N-acetyl-P-benzoquinoneimine).(Kapur V. *et al*, 1994)

Most of the people consume Ethenol which causes the liver damage by the mechanism i.e.-alcohol consumption causes increased activity of TNF- α is the increased intestinal permeability due to liver disease. This facilitates the absorption of the gut-produced endotoxin into the portal circulation. The Kupffer cells of the liver then phagocytose endotoxin, stimulating the release of TNF- α . TNF- α then triggers apoptotic pathways through the activation of caspases, resulting in cell death. (Menon KV, *et al*, 2001)

The extract contains Flavonoid, total phenol compounds and Nitrogenous compounds show the significance role in liver disease which was administered orally 300mg/kg (according to OECD-423) to whister rat single times in a day. Silymarine (25mg/kg) was selected as the standard drug which is well known Hepatoprotective drug.

Significance was observed by evaluating the SGOT, SGPT, TB, and ALP, (appear lowering). Histopathology examinations of liver section support the study.

AST predominantly found in mitochondria of hepatocytes. ALT is more specific to liver, and thus is a better parameter for detecting liver injury. Serum ALP and bilirubin is also associated with liver cell damage. The ALT, AST and ALP activity and serum bilirubin level are largely used as most common biochemical markers to evaluate liver injury [Kozer E, *et al*, 2003].

Administration of paracetamol caused a significant elevation of enzymes level such as AST, ALT, ALP and bilirubin level has been attributed to the damage structural integrity of liver, because they are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity [Sallie R., *et al*, 1991]

In this experiment it was founded that *Abelmoschus moschatus* seed extract 300mg/kg show the significant result in the paracetamol induced hepatotoxicity as the value of SGOT, SGPT, ALP and TB, were found significant ($p < 0.001$) compared with paracetamol treated and less significant ($p < 0.1-0.05$) when compared with only vehicle treated statically by one way ANOVA followed by Tukey's multiple comparison test.

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